APCRA New Case Study Proposal template

1. Title of Case Study: **Substantiating Chemical Categories with Omics-derived Mechanistic Evidence (SuCCess)**

2. Lead organization:

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Organization:	ECHA

3. Potential Collaborator(s):

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Organization:	Environment and Climate Change Canada Japanese Ministry of the Environment

4. Problem to be addressed by case study:

   Grouping and read-across are frequently used for human health and environmental endpoints, for example, developmental and reproductive toxicity. The quality of this hazard assessment methodology, however, still needs to improve. In particular, from ECHA's perspective, registrants often do not provide enough scientific evidence to support their read-across case. Critical to the success of read-across is the formation of a category (or group) of similar substances that are believed to share the same mechanism of action. Traditionally, substances have been grouped based only on their structural parameters, not using any mechanistic evidence of toxicity pathways.

5. Aim/Purpose of case study:

This case study will evaluate the effectiveness of NAMs, specifically omics technologies used in conjunction with third-wave machine learning, to derive molecular data for mechanism-driven substance grouping. Specifically, the similarities/dissimilarities of the molecular responses to several substances will be used to substantiate (or not) a grouping hypothesis derived from traditional QSAR approaches.

In addition, the case study will explore the capability of a *concentration x time* experimental design as well as a *multi*-omics strategy (combining transcriptomics and metabolomics) for determining the "sameness" of the molecular responses to substances.

This case study is focused on EcoNAMs, and will investigate and group the molecular responses of *Daphnia magna* to the test substances. A series of practical considerations have been developed for the selection of the test substances, and as a result a group of azo dyes have been prioritised for study by ECHA.

A further purpose of the case study is to trial the OECD Omics Reporting Frameworks for transcriptomics and metabolomics (dependent on their rate of development) and more generally to familiarise APCRA scientists with multi-omics data types and machine learning approaches.

6. Main Steps/General Timeframe:

(a) Substance selection: conduct structure based grouping (i.e. OECD QSAR Toolbox) to generate a similarity/dissimilarity map of a large number of azo dyes (often sub-divided into water soluble (azo acidic, azo direct and azo reactive) and water insoluble (azo disperse, azo mordant and azo solvent) dyes). Approximately ten substances will be selected for experimental investigation to test specific hypotheses on structural "sameness", focusing on one structurally similar group of azo dyes and likely including up to two known "outliers" (structurally). APCRA partners could contribute to the substance selection phase and are encouraged to propose a second group of substances for testing.

(b) Dose range-finding experiments in *Daphnia*: conduct range-finding studies with *Daphnia* to determine the "equi-effective" concentrations (for each of the substances tested) to be used in the subsequent omics studies. These concentrations will be anchored to an established apical endpoint in *Daphnia*, for example using the benchmark dose (lower confidence limit) (BMDL) for 5% acute immobilisation or chronic reproductive fitness. APCRA partners could contribute to refining this experimental design and the analysis of the range-finding data.

(c) Exposures, transcriptomics and metabolomics analyses: *Daphnia* will be exposed to each substance and their transcriptomic and metabolomic signatures measured across several time points and three concentrations. Third-wave machine learning technologies - artificial intelligence modalities that combine the accuracy of deep neural networks with the interpretability of statistical learning – will be used to integrate metabolomics and transcriptomics data to provide mechanistic evidence-based groupings predicated on shared molecular signatures. We will ask if justification of the category (originally based on structure) can be supported by the presence of common molecular signatures.

(d) Elucidating mechanism: we will attempt to identify perturbed molecular signatures by mapping to human genes using a KnowledgeBase (such as Ingenuity Pathway Analysis). This will attempt to link perturbed molecular biomarkers (or Key Events) to pathways to adverse effects. Then we will ask: Are perturbed pathways predictive of adverse effects? Do they identify a known pathway associated with cancer or other important biological condition (potentially triggering more extensive testing)? What are the mechanistic similarities/differences between the substances in the group and can the substances with the highest concern be identified?

(e) Cross species: if a “hit” is observed in both *Daphnia* and human, then the hypothesis is that this toxicity pathway is conserved in both invertebrate and vertebrate animals. Extending the species tested beyond *Daphnia* could help to identify and localise the toxicity pathways within the phylogenetic tree of life. APCRA partners are encouraged to could contributing a further test organism to the study.

7. Expected Regulatory Application/Impact of Case Study:

To demonstrate the capability of an (Eco)NAM workflow that generates and uses omics-derived molecular signatures to improve the reliability of chemical categories, as a basis for more robust read-across; i.e. to substantiate the overall mechanistic consistency of the category in question. Furthermore, having attempted to identify the predominant MoA/pathways, to seek to demonstrate the potential relevance of this approach for human health.